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Actions of Ethanol on Voltage-Sensitive Sodium Channels: Effects on Neurotoxin-Stimulated Sodium Uptake in Synaptosomes

MICHAEL J. MULLIN[1] and WALTER A. HUNT
Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, National Naval Medical Center, Bethesda, Maryland
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ABSTRACT

Exposure of rat brain synaptosomes to ethanol *in vitro* reduced the neurotoxin-stimulated uptake of $^{22}Na^+$. This effect of ethanol was concentration-dependent, occurred with concentrations of ethanol achieved *in vivo* and was fully reversible. The inhibitory effect of ethanol on neurotoxin-stimulated sodium uptake was due to a decrease in the maximal effect of the neurotoxins. Ethanol reduced the rate of batrachotoxin-stimulated sodium uptake when measured at 3, 5 and 7 but not 10 or 20 sec after the addition of $^{22}Na^+$. In a series of aliphatic alcohols, there was a good correlation between potency for inhibition of batrachotoxin-stimulated $^{22}Na^+$ uptake and the membrane/buffer partition coefficient, suggesting that a hydrophobic site in the membrane was involved in the action of the alcohols. Ethanol did not affect the scorpion venom-induced enhancement of batrachotoxin-stimulated sodium uptake. The inhibitory potency of tetrodotoxin was also unaffected by ethanol. These results demonstrate that ethanol has an inhibitory effect on neurotoxin-stimulated sodium influx occurring in voltage-sensitive sodium channels of brain tissue.

The exact mechanisms by which ethanol causes depression of the CNS and the subsequent behavioral manifestations of intoxification remain undefined. In recent years, a great deal of research has focused on the effects of ethanol on the physical properties of artificial and biological membranes (Seeman, 1972; Goldstein *et al.*, 1980). Through the use of techniques such as electron paramagnetic resonance (Chin and Goldstein, 1977a) and fluorescence spectroscopy (Harris and Schroeder, 1981), it has been demonstrated that pharmacologically relevant concentrations of ethanol *in vitro* cause disordering of membrane lipids as inferred from the measurements of the properties of molecular probes inserted into membranes. By using a variety of molecular probes that insert at different depths in the membrane, it has been shown that the fluidizing effect of ethanol is greater at the hydrophobic inner core than at the superficial membrane surface (Chin and Goldstein, 1981; Harris and Schroeder, 1981). This is somewhat surprising because ethanol is a relatively small, neutral, hydrophilic molecule.

Further evidence that membrane disordering is involved in the intoxicating effects of ethanol has been based on a number of studies that have provided genetic (Goldstein *et al.*, 1982), pharmacological (Lyon *et al.*, 1981) and temporal (Chin and Goldstein, 1977b) correlations between *in vivo* effects and the membrane disordering effect of ethanol *in vitro*. However, because the magnitude of membrane lipid disordering by ethanol is relatively small (Chin and Goldstein, 1977a), it is probable that membrane-mediated biological responses would involve functional entities in membranes. Consequently, ethanol would be expected to interact directly or indirectly with these entities, so that alterations in the functional properties of membrane proteins may also play an important role in the changes in neuronal activity and synaptic function associated with ethanol-induced depression of the CNS. Alterations in lipid fluidity are known to influence the activities of membrane proteins (Lenaz, 1977) and this may represent a mechanism by which the lipid disordering effect of ethanol could be translated into the biochemical and behavioral effects of ethanol.

In the present study, a basic functional unit related to neuronal excitation was investigated with respect to its possible involvement in the actions of ethanol on the brain. An important mechanism in the control of neuroexcitability is the regulation of ion movements at the level of the excitable membrane. Using the squid giant axon, Hodgkin and Huxley (1952) demonstrated that the changes in membrane voltage associated with an action potential were due to a transient increased

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ABBREVIATIONS: CNS, central nervous system; TTX, tetrodotoxin; BTX, batrachotoxin; VER, veratridine; ScV, scorpion venom; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

permeability to sodium followed by an increased permeability to potassium. Apparently, separate channels are utilized by sodium and potassium ions (Hille, 1970; Ulbricht, 1977). The inward movement of sodium ions during excitation occurs through voltage-sensitive sodium channels. The sodium channels that traverse the excitable membrane are composed of complex glycoproteins with multiple polypeptide subunits and contain three distinct receptor sites for various neurotoxins (Catterall, 1980). One receptor site, thought to be located on the extracellular side of the channel (Narahashi, 1966), binds the specific inhibitors saxitoxin and TTX which inhibit the inward movement of sodium (Ritchie and Rogart, 1977). The second neurotoxin receptor site binds the lipid soluble toxins, BTX and VER, which cause persistent activation of sodium channels by blocking the process of channel inactivation and by shifting the voltage-dependence of channel activation to more negative membrane potentials. The third neurotoxin receptor binds small polypeptide toxins present in sea anemone and ScVs. The polypeptide toxins slow channel inactivation and also enhance the effects of BTX and VER. Because the neurotoxins bind to their receptor sites with high affinity and specificity, they have been widely used as chemical tools to study the structure and functional properties of voltage-sensitive sodium channels in a variety of excitable membranes (Narahashi, 1974; Catterall, 1982).

We have studied the effects of ethanol *in vitro* on the functional properties of voltage-sensitive sodium channels in whole rat brain synaptosomes. Ion flux measurements were used as an estimate of sodium ($^{22}Na^+$) ion permeability to assess the function of synaptosomal sodium channels.

Methods

Animals and chemicals. Male Sprague-Dawley rats (200–400 g) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and were housed two per cage with free access to water and standard laboratory chow before being used for the experiments. Chemicals and suppliers were as follows: ScV (Leiurus quinquestriatus), TTX and VER from Sigma Chemical Co. (St. Louis, MO); carrier-free $^{22}NaCl$ from New England Nuclear (Boston, MA). BTX was kindly supplied by Dr. John Daly (National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD). All other chemicals were obtained from commercial sources and were of analytical grade.

Preparation of synaptosomes. A crude synaptosomal (P_2) fraction was prepared by a modification of the method of Gray and Whittaker (1962). Rats were decapitated and the whole brains were removed and homogenized in ice-cold 0.32 M sucrose and 5 mM K_2HPO_4, pH 7.4 (10 ml/g wet wt.), with 10 strokes of a motor-driven Teflon-glass homogenizer. The homogenate was then centrifuged at 1000 × *g* for 10 min. The resulting supernatant was then centrifuged at 17,000 × *g* for 60 min. The final pellet was resuspended in ice-cold incubation buffer (8–10 ml/brain) containing (millimolar): KCl, 5.4; $MgSO_4$, 0.8; glucose, 5.5; HEPES-Tris (pH 7.4), 50; and choline chloride, 130. Ten strokes of a loose fitting glass-glass homogenizer were used to resuspend the final pellet. Synaptosomes were kept on ice and were used immediately after preparation.

Measurement of $^{22}Na^+$ uptake. Synaptosomal sodium uptake was determined by a modification of the method of Tamkun and Catterall (1981). Aliquots (50 μl) of the synaptosomal suspension were preincubated at 36°C for 2 min, except in the time course experiments, with incubation buffer or incubation buffer containing the indicated concentration of ethanol. Immediately after the preincubation with ethanol, the indicated concentration of activator toxin (BTX or VER) was added and the samples were incubated for 10 min at 36°C. After 10 min the samples were diluted with a solution containing (final concentration) the indicated concentration of toxin (millimolar): KCl, 5.4; $MgSO_4$, 0.8; glucose, 5.5; HEPES-Tris (pH 7.4), 50; choline chloride, 128; NaCl, 2; ouabain, 5; and 1.3 μCi of carrier-free $^{22}NaCl$ per ml and the indicated concentration of ethanol. After a 5-sec incubation (except where noted), uptake was terminated by the addition of 3 ml of an ice-cold wash solution containing (millimolar): choline chloride, 163; $MgSO_4$, 0.8; $CaCl_2$, 1.8; HEPES-Tris (pH 7.4), 5; and bovine serum albumin, 1 mg/ml. The mixture was filtered rapidly under vacuum through an Amicon 0.45-μm cellulose filter (Amicon, Lexington, MA) and the filters were washed twice with 3 ml of wash solution. The entire halt-filter-wash cycle took less than 10 sec to complete. The filters were placed in scintillation vials, 15 ml of scintillation cocktail were added and filter radioactivity was determined by liquid scintillation spectrometry. The data are presented as corrected specific uptake after subtraction of nonspecific uptake (TTX, 1 μM present in incubation and uptake buffers).

Data analysis. Double reciprocal analysis of the data was performed as described by Catterall (1975) using a modified Michaelis-Menton equation of the form:

$$v = VA/(K_{0.5} + A)$$

where v is the uptake rate at various activator toxin concentrations A, V is maximal uptake rate and $K_{0.5}$ is the apparent dissociation constant of the activator toxin. Statistical analysis was performed using Student's t test for paired samples. Multiple comparisons with a control were done by analysis of variance and Dunnett's test (1964).

Other methods. Drug concentrations in the aqueous and membrane phases were calculated as described by Lyon *et al.* (1981). The membrane/buffer partition coefficients were derived from the data of McCreery and Hunt (1978). Solutions of ScV were prepared according to Catterall (1976). A stock solution of BTX was prepared in absolute ethanol and aliquots were diluted in the appropriate buffer. The final concentration of ethanol in the assay from the addition of BTX was never greater than 0.13 mM. Protein concentrations were determined by the method of Lowry *et al.* (1951). Bovine serum albumin was used as the protein standard.

Results

Preincubation of whole brain synaptosomes with ethanol *in vitro* caused a concentration-dependent inhibition of BTX-stimulated $^{22}Na^+$ uptake (fig. 1). Over the concentration range of ethanol used, the reduction in $^{22}Na^+$ uptake was a linear function of the concentration of ethanol ($r = -0.95$). The inhibitory effect of ethanol occurred at pharmacologically relevant concentrations of ethanol and was fully reversible when ethanol was removed from the sample by washing and centrifugation (fig. 2). Neither the nonspecific (1 μM TTX present) nor the passive, unstimulated (no toxins present) uptake of $^{22}Na^+$ were affected by ethanol *in vitro* (data not shown).

The effects of ethanol on $^{22}Na^+$ uptake were also studied over a range of concentrations of the alkaloid toxins BTX and VER. There was a 6-fold increase in synaptosomal sodium uptake when the concentration of BTX was raised from 0.1 to 5 μM. Ethanol (100 and 400 mM) inhibited $^{22}Na^+$ uptake in synaptosomes exposed to more than 0.1 μM BTX (fig. 3). When VER was used to activate sodium channels and promote $^{22}Na^+$ uptake, a similar effect of ethanol was observed, as shown in figure 4. In order to assess indirectly the interaction of ethanol with the channel receptor site for BTX and VER, we performed a double reciprocal analysis of concentration-effect curves for BTX- and VER-stimulated $^{22}Na^+$ uptake in the absence (control) and presence of two concentrations of ethanol (100 and 400 mM). The data from the double reciprocal analysis are

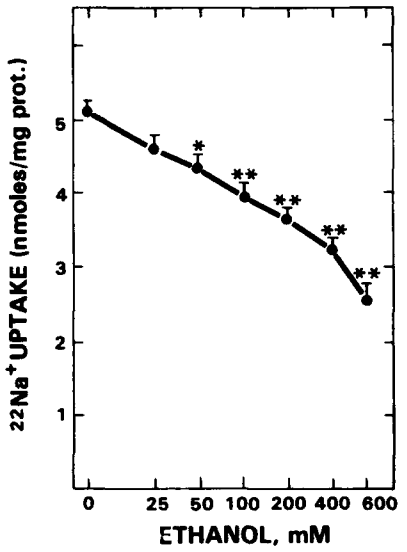

Fig. 1. Concentration-effect curve for inhibition of BTX-stimulated $^{22}Na^+$ uptake. Triplicate samples of whole brain synaptosomes were preincubated for 2 min with the indicated concentration of ethanol followed by a 10-min incubation with 1 μM BTX. Symbols represent the means ± S.E.M., $N = 5$ experiments. The corrected specific uptake of $^{22}Na^+$ during a 5-sec period is shown on the ordinate. The concentration of ethanol is shown on the abscissa, log scale. *$P < .01$ (Dunnett's test) compared to uptake measured in the absence of ethanol. prot., protein.

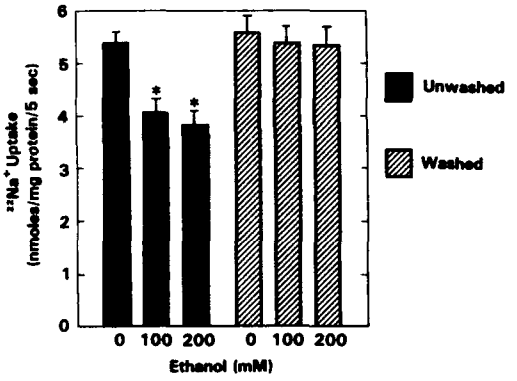

Fig. 2. Reversibility of the inhibitory effect of ethanol on BTX-stimulated $^{22}Na^+$ uptake. After a 2-min preincubation with ethanol, one-half of the samples were centrifuged (1000 × g for 5 min), washed and resuspended in incubation buffer. BTX (1 μM) was added, samples were incubated at 36°C for 10 min and $^{22}Na^+$ uptake was measured as described. The data are expressed as the means ± S.E.M., $N = 4$ experiments. The corrected specific uptake of $^{22}Na^+$ is shown on the ordinate. The concentration of ethanol is shown on the abscissa. *$P < .01$ (Dunnett's test) compared to uptake in control samples.

summarized in table 1. When sodium channels were activated by BTX, ethanol acted as a noncompetitive inhibitor as the maximum uptake of $^{22}Na^+$ was reduced with no change in the concentration of BTX required for 50% of maximum uptake ($K_{0.5}$). The effects of ethanol on VER-stimulated $^{22}Na^+$ uptake are somewhat more difficult to interpret. Clearly, ethanol significantly reduced the maximum effect of VER. In addition, ethanol reduced the $K_{0.5}$ values for VER but the difference from control was not significant.

The time courses for $^{22}Na^+$ uptake with 1 μM BTX alone and with 1 μM BTX plus 200 mM ethanol are illustrated in figure 5. In the presence of ethanol, the BTX-stimulated uptake of $^{22}Na^+$ was significantly reduced at uptake times of 3, 5 and

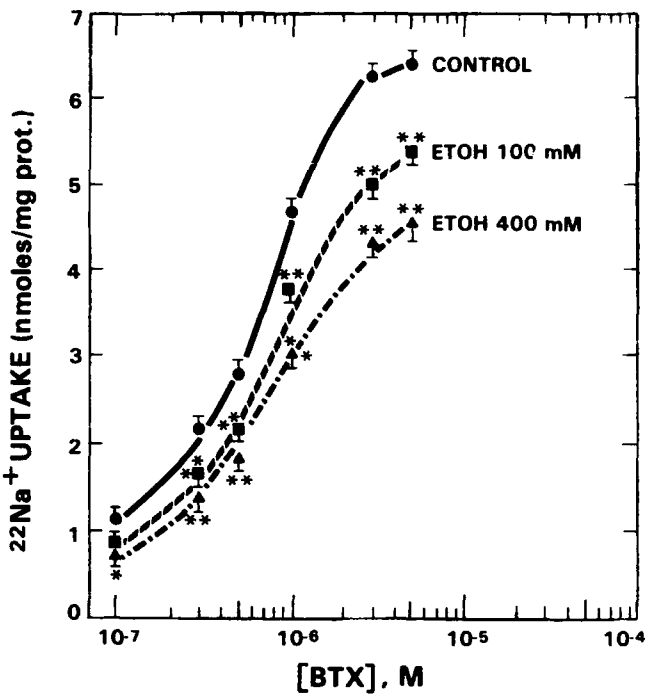

Fig. 3. Effect of ethanol on concentration-effect curve for BTX-stimulated $^{22}Na^+$ uptake. Synaptosomes were preincubated with buffer only or buffer containing ethanol (100 and 400 mM) for 2 min. The indicated concentration of BTX was added and samples were incubated for an additional 10 min and $^{22}Na^+$ uptake was measured for 5 sec as described under "Methods." Symbols represent the means ± S.E.M., $N = 6$ experiments. *$P < .05$; **$P < .01$ (Dunnett's test) compared to corresponding control. prot., protein.

7 sec, but not at 10 or 20 sec. Thus, the effect of ethanol on $^{22}Na^+$ uptake is an inhibitory effect on the initial rates of $^{22}Na^+$ uptake. We were unable to measure $^{22}Na^+$ uptake at uptake times shorter than 3 sec with acceptable precision. The results in table 2 demonstrate that the duration of the preincubation period with ethanol was not an important determinant of the inhibitory effect of ethanol. An ethanol concentration of 200 mM reduced the specific uptake to 77.2, 79.3, 74.5 and 77.4% after 0, 0.5, 2 and 10 min, respectively. Thus, the onset of action was immediate and was unchanged over the time periods studied.

The effect of increasing lipid solubility on potency for inhibition of BTX-stimulated $^{22}Na^+$ uptake was studied for a series of aliphatic alcohols. Membrane-buffer partition coefficients were used to calculate the concentration of each alkanol that would result in a similar molar concentration in the nonaqueous (membrane) phase (Lyon *et al.*, 1981). Concentration-effect curves were constructed for each alkanol as percentage of control uptake *vs.* log of the alkanol concentration. Linear regression analysis was used to determine the correlation coefficients, slopes and the concentration of alkanol that inhibited control uptake by 50% (IC_{50}). These values are shown in table 3. For each alkanol tested, the reduction in $^{22}Na^+$ uptake was a linear function of the concentration of alkanol.

The IC_{50} and membrane/buffer partition coefficient values for each alkanol were plotted on a log-log scale (fig. 6). There was a good correlation ($r^2 = 0.997$) between the two parameters, indicating that the ability to partition into a hydrophobic region of the membrane was an important determinant of the potency for inhibition of BTX-stimulated $^{22}Na^+$ uptake.

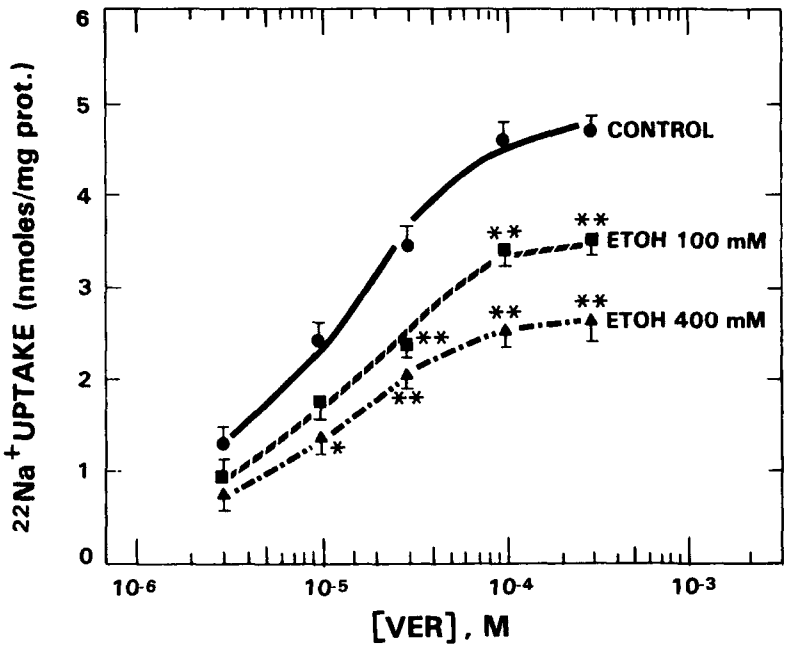

Fig. 4. Effect of ethanol on concentration-curve for VER-stimulated $^{22}Na^+$ uptake. Synaptosomes were preincubated with buffer only or buffer containing ethanol (100 and 400 mM) for 2 min. The indicated concentration of VER was added, samples were incubated for an additional 10 min and $^{22}Na^+$ uptake was measured for 5 sec as described under "Methods." Symbols represent the means ± S.E.M., $N = 4$ experiments. *$P < .05$; **$P < .01$ (Dunnett's test) compared to corresponding control. prot., protein.

TABLE 1

Double reciprocal analysis of alkaloid toxin activation of sodium channels

Alkaloid Toxin	Ethanol Conc.	N	V[a]	$K_{0.5}$[a]
	mM		*nmol/mg protein/5 sec*	*μM*
BTX	0	6	6.43 ± 0.27[b]	0.430 ± 0.024
BTX	100	6	4.84 ± 0.15*	0.439 ± 0.042
BTX	400	6	4.09 ± 0.10*	0.431 ± 0.022
VER	0	4	4.62 ± 0.22	9.33 ± 0.70
VER	100	4	3.30 ± 0.29*	7.83 ± 0.94
VER	400	4	2.50 ± 0.12*	6.58 ± 0.63

[a] Values for maximum uptake (V) and $K_{0.5}$ were calculated using a modified Michaelis-Menton equation as described under "Methods."
[b] Values are means ± S.E.M. N = number of experiments.
* Significantly different from control, $P < .01$.

ScV alone does not promote sodium influx. However, ScV enhances alkaloid toxin-stimulated $^{22}Na^+$ uptake, presumably by an allosteric mechanism (Catterall, 1980; Tamkun and Catterall, 1981). The effect of ethanol on the ScV-BTX interaction is shown in figure 7. In control samples, ScV at concentrations of 1 and 10 μg/ml increased BTX-stimulated $^{22}Na^+$ uptake by 26.5 ± 1.6 and 62.5 ± 1.7%, respectively. In the presence of ethanol, a similar enhancement of BTX-stimulated $^{22}Na^+$ uptake was noted (1 μg/ml, 27.3 ± 3.8; 10 μg/ml, 54.2 ± 3.8%). Thus, ethanol does not have a significant effect on the allosteric interaction of ScV and BTX.

The effect of ethanol on the inhibition of BTX-stimulated $^{22}Na^+$ uptake by TTX is shown in figure 8. An ethanol concentration of 200 mM did not affect the potency of the specific inhibitor TTX. The concentration of TTX necessary for a 50% reduction in BTX-stimulated $^{22}Na^+$ uptake in control samples was 12.72 ± 0.61 nM. In the presence of ethanol, a similar value (12.60 ± 0.59 nM) was measured.

Discussion

Incubation of synaptosomes with ethanol *in vitro* caused a significant inhibition of the initial rates of neurotoxin-stimulated sodium uptake. This effect of ethanol was concentration-

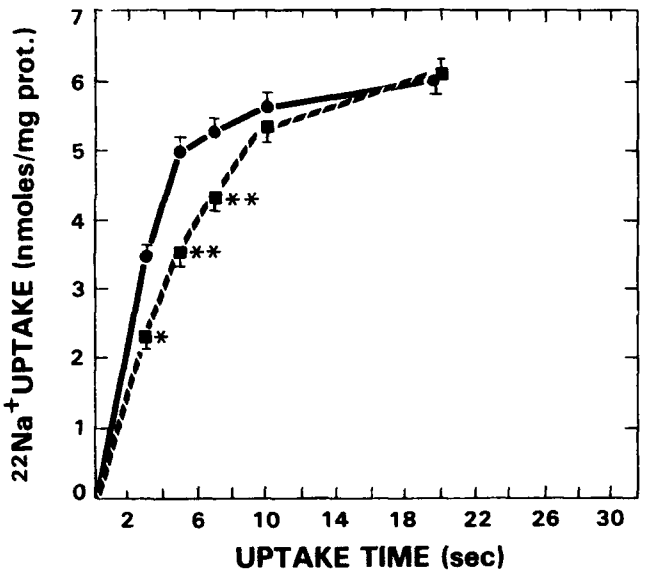

Fig. 5. Effect of ethanol on the time course of BTX-stimulated $^{22}Na^+$ uptake. Synaptosomes were preincubated with buffer only or buffer containing ethanol (200 mM) for 2 min. BTX (1 μM) was added and samples were incubated for an additional 10 min and $^{22}Na^+$ uptake was measured for the indicated time. Symbols represent the means ± S.E.M., $N = 5$ experiments. *$P < .05$; **$P < .01$ (paired t test) compared to corresponding control. prot., protein.

TABLE 2

Effect of preincubation time with ethanol on inhibition of BTX-stimulated $^{22}Na^+$ uptake; duration of preincubation[a]

	0 min	0.5 min	2.0 min	10 min
Control	4.72 ± 0.35[b]	4.82 ± 0.39	5.22 ± 0.17	4.65 ± 0.25
Ethanol, 200 mM	3.64 ± 0.39*	3.82 ± 0.38*	3.89 ± 0.20*	3.60 ± 0.21*

[a] Refers to the duration of preincubation of tissue with buffer or buffer containing ethanol (200 mM) before the addition of BTX (1 μM).
[b] Values are means ± S.E.M., $N = 3$ experiments, units are nanomoles of $^{22}Na^+$ per milligram of protein per 5 sec.
* Significantly different from corresponding control, $P < .01$.

TABLE 3

Effects of aliphatic alcohols *in vitro* on BTX-stimulated $^{22}Na^+$ uptake

Alcohol	$P_{m/b}$[a]	IC_{50}[b]	Slope[b]	r^2
Ethanol	0.096	582.8 ± 51.8	−71.1 ± 7.2	0.980
Propanol	0.438	104.0 ± 13.6	−72.8 ± 6.9	0.983
Butanol	1.52	35.9 ± 5.4	−65.1 ± 9.2	0.993
Pentanol	5.02	6.6 ± 0.6	−55.9 ± 6.6	0.940
Hexanol	21.4	1.2 ± 0.06	−60.0 ± 9.6	0.936

[a] Membrane/buffer partition coefficient ($P_{m/b}$) values are from McCreery and Hunt (1978).

[b] Values are the means ± S.E.M., N = 4–8 experiments. In each experiment, three or four concentrations of each alkanol were tested. IC_{50}, the concentration of each alcohol necessary to reduce control BTX-stimulated uptake by 50%. Values were derived from linear regression analysis of percentage of control uptake *vs.* log [alkanol].

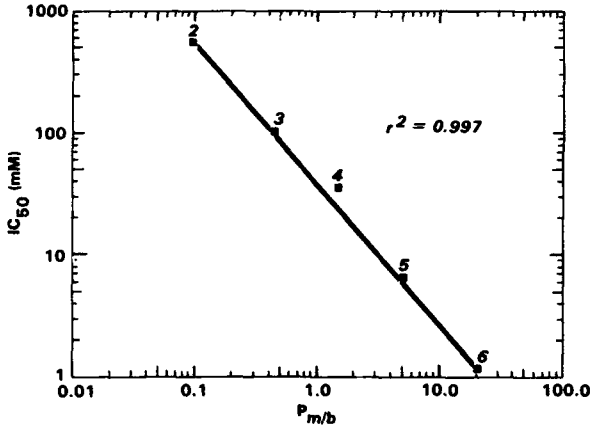

Fig. 6. Correlation of the inhibitory potency of aliphatic alcohols on BTX-stimulated $^{22}Na^+$ uptake with their membrane/buffer partition ($P_{m/b}$) coefficient. The concentration of each alcohol that inhibits BTX-stimulated $^{22}Na^+$ uptake by 50% (IC_{50}, millimolar) is presented on the ordinate. The $P_{m/b}$s are shown on the abscissa. The number above each symbol represents the chain length of the alcohol: 2, ethanol; 3, *n*-propanol; 4, *n*-butanol; 5, *n*-pentanol; and 6, *n*-hexanol. The line was fit by linear regression analysis with a correlation coefficient, r^2 = 0.997. See table 3 for details.

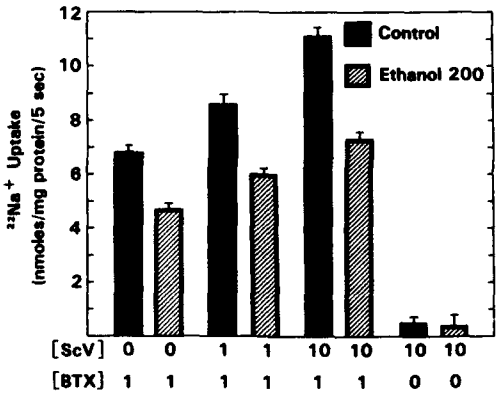

Fig. 7. The effect of ethanol *in vitro* on the BTX-ScV interaction. An aliquot of synaptosomes was preincubated for 2 min with buffer (control) or buffer containing ethanol. The indicated concentrations of BTX and ScV were added and $^{22}Na^+$ uptake was measured as described. The units of concentration for ScV and BTX were micrograms per milliliter and micromolar, respectively. The data are presented as the means ± S.E.M., N = 4 experiments.

dependent, occurred with pharmacologically relevant (50 mM) concentrations of ethanol and was fully reversible when ethanol was removed from the system. In addition, there was a good correlation between potency for inhibition of BTX-stimulated

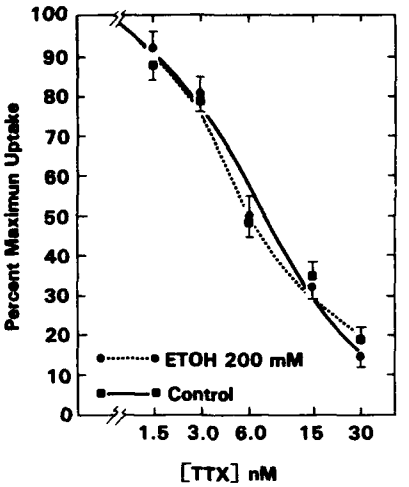

Fig. 8. Effect of ethanol on the inhibition of BTX-stimulated $^{22}Na^+$ uptake by TTX. Triplicate samples of whole brain synaptosomes were preincubated for 2 min with buffer (control) or with buffer containing ethanol. The indicated concentration of TTX was added and 3 min later BTX (1 μM) was added and all samples were incubated for an additional 10 min. Symbols represent the means ± S.E.M., N = 4 experiments. Maximum uptake for control and ethanol samples was 6.50 ± 0.13 and 4.33 ± 0.13 nmol of $^{22}Na^+$ per mg of protein per 5 sec, respectively.

$^{22}Na^+$ uptake and the membrane/buffer partition coefficients for a series of aliphatic alcohols, suggesting that a hydrophobic area of the sodium channel microenvironment was involved in the action of the alkanols. Inasmuch as the neurotoxin-stimulated uptake of sodium was blocked by the specific inhibitor TTX with a $K_{0.5}$ of approximately 13 nM, it appears that the neurotoxin-stimulated sodium uptake that was inhibited by ethanol occurred through voltage-sensitive sodium channels in synaptosomes (Catterall, 1980). Our results with ethanol are consistent with the work of Harris (1984) which showed that a variety of intoxicant anesthetics agents reduced neurotoxin-stimulated sodium uptake.

In the CNS, ionic channels for calcium, potassium and sodium are involved intimately in the control of excitability and each plays an essential role in signal transduction and information processing (Catterall, 1984). At the present time, the structural and functional properties of the voltage-sensitive sodium channels are understood most completely. This is due in part to the existence of a variety of neurotoxins that can be used as tools to study the sodium channel (Narahashi, 1974). In this regard, BTX has been defined as a full agonist (Catterall, 1980; Tamkun and Catterall, 1981) because it activates a larger number of sodium channels and is also more potent ($K_{0.5}$ value is smaller) than the partial agonist VER.

There appeared to be slight differences in the effect of ethanol when different neurotoxins were used to stimulate sodium uptake. When BTX was used to stimulate sodium uptake, the maximum effect of BTX was reduced by ethanol with no change in the $K_{0.5}$ value. Also, the minimum effective concentration of ethanol was 50 mM and the concentration of ethanol required for 50% inhibition (IC_{50}) was approximately 583 mM. VER-stimulated sodium uptake was more sensitive to inhibition by ethanol because the minimum effective concentration of ethanol was 25 mM and the IC_{50} value was approximately 345 mM (Mullin and Hunt, 1984). Ethanol reduced the maximum effect of VER and there was also a clear trend toward reducing the $K_{0.5}$ value of VER. It would be desirable to perform

more experiments with VER to investigate further this point, but during the course of this study VER became unavailable commercially. Even though it is well known that BTX and VER compete for the same binding site in the channel (Catterall, 1975), the mechanism by which each neurotoxin stimulates sodium uptake may be slightly different because of other properties of the toxins (Miller, 1983; Tanaka *et al.*, 1983).

The action of ethanol on the voltage-sensitive sodium channels in synaptosomes appears to be somewhat selective for the site that binds BTX and VER. Ethanol did not interfere with the allosteric interaction that occurred between BTX and ScV. Similarly, the presence of ethanol did not alter the concentration of TTX required to inhibit BTX-stimulated sodium uptake by 50%. However, it must be noted that these findings concerning the effects of ethanol on neurotoxin receptor sites I (TTX) and III (ScV) are based on indirect measurements. Direct measurement using radiolabeled neurotoxins should clarify these points.

Recent studies with fluorescent derivatives of sodium channel neurotoxins (Angelides and Nutter, 1983, 1984) in conjunction with published biochemical evidence (Hartshorne and Catterall, 1981, 1984; Ellisman *et al.*, 1982) have advanced our knowledge of the molecular arrangement of the functional components of the sodium channel. Angelides and Nutter (1984) have proposed a model wherein the TTX binding site resides in a highly polar, hydrophilic area at the extracellular side of the membrane. The binding site for ScV may be located in a more hydrophobic region that is not lipid in nature and may extend 15 A into the cell membrane. The final neurotoxin receptor site (for BTX and VER) is placed in a hydrophobic area directly interacting with the subunit of the channel and the interior of the lipid bilayer. Thus, in this model, the BTX/VER binding site is located in the area of the membrane in which the fluidizing effect of ethanol is greatest (Harris and Schroeder, 1981). An explanation for the observed effects of ethanol on neurotoxin-stimulated sodium uptake may involve changes in the fluidity of the neuronal membrane as a number of other membrane perturbants inhibit sodium uptake with potency for inhibition of sodium uptake being related to lipid solubility (Harris, 1984). Ethanol may alter the arrangement of membrane lipids or hydrophobic proteins in a specific area of the sodium channel microenvironment. In addition, disruption of important lipid-protein interactions by ethanol may result in suboptimal conditions that would adversely affect sodium channel function. Further studies of sodium channel function in systems in which the lipid and protein components are more tightly controlled may answer some of these questions.

It is difficult to determine how important the effect of ethanol on sodium channels is to the intoxicating effect of ethanol. In this study and that of Harris and Bruno (1985), an ethanol concentration of 50 to 100 mM was required to produce a significant inhibitory effect. Previous work from our laboratory demonstrated that an ethanol concentration of 25 mM which is commonly achieved *in vivo* and is associated with moderate intoxication was sufficient to cause a significant inhibition of VER-stimulated $^{22}Na^+$ uptake (Mullin and Hunt, 1984). Additionally, it appears that brain regions differ in sensitivity to this effect of ethanol (Harris and Bruno, 1985).

We have used an ion flux assay to study the effects of ethanol on sodium channel function in synaptosomes in which electrophysiological methods are not practical. Because it is necessary to use toxins to activate the sodium channels, one must consider the possibility that ethanol is interfering with the binding of the toxins to their receptors in the channel. This could explain the results we have presented. This question is currently under study in our laboratory using radiolabeled toxins.

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Send reprint requests to: Dr. Michael J. Mullin, Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Naval Medical Command, National Capital Region, Bethesda, MD 20814-5011.